

# **Clinical profile and genetic studies in Recurrent Acute Pancreatitis : A five year study.**

**A dissertation submitted in part fulfillment of DM  
(Gastroenterology) examination of the Tamil Nadu Dr.MGR  
Medical University , Chennai to be held in August 2008.**

## **CERTIFICATE**

This is to certify that this dissertation entitled **Clinical profile and genetic studies in Recurrent Acute Pancreatitis : A five year study** is a bonafide work done by Dr. Sajith.K.G in partial fulfillment of the rules and regulations for DM (Gastroenterology) examination of Tamil Nadu Dr.MGR Medical University , to be held in August 2008.

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# INTRODUCTION

Numerous studies have been conducted on acute and chronic pancreatitis , but only few have focused on recurrent acute pancreatitis(RAP). Recurrent acute pancreatitis may be due to biliary disease, alcohol, metabolic factors (hypercalcemia, hypertriglyceridemia), drugs, trauma, sphincter of Oddi dysfunction, pancreas divisum and pancreatic carcinoma<sup>(1)</sup> Evaluation fails to detect the cause in 10 – 30% of patients , and these patients are labelled as idiopathic recurrent acute (IAP) pancreatitis(IRAP). Further evaluation and therapy is important because more than 50% of untreated patients with RAP experience recurrent episodes that can lead to chronic pancreatitis<sup>(2)</sup>

Mutations in cationic trypsinogen gene (PRSS1) , SPINK1 gene ,cystic fibrosis transmembrane conductance regulator gene (CFTR) and Cathepsin B gene have been demonstrated in acute and chronic pancreatitis<sup>(3-6)</sup>. It is possible that genetic mutations may be the cause of pancreatitis in patients now labelled as Idiopathic Recurrent Pancreatitis. Few patients with gall stones or alcoholism develop RAP. It is therefore conceivable that genetic mutations enhance susceptibility to RAP in patients with other predisposing factors. RAP may be a complex disease process resulting from an interplay of genetic susceptibilities and environmental factors.

A systematic study on etiology ,clinical features , response to therapy and outcome in patients with RAP has not been carried out in India. We need to get data on the following:

- (a) What are the common etiological factors causing RAP?
- (b) What percentage of patients have Idiopathic RAP (IRAP)?

- (c) What percentage of patients initially diagnosed as RAP develop chronic pancreatitis on follow up?
- (d) Response to therapy-Medical, Endoscopic .
- (e) Morbidity and mortality.

Therefore , the aim of the present study was: (a) to study the clinical profile, efficacy of medical / endoscopic therapy and outcome of patients with RAP.(b) assess the prevalence of genetic mutations in a subset of patients with RAP.

## **AIMS AND OBJECTIVES**

1. To study the clinical profile, efficacy of medical / endoscopic therapy and outcome of recurrent acute pancreatitis.
2. To assess the prevalence of genetic mutations in recurrent acute pancreatitis.

## REVIEW OF LITERATURE

### DEFINITIONS:

Acute Pancreatitis: is defined as an acute inflammatory disease of the pancreas presenting with abdominal pain and usually associated with elevated pancreatic enzymes in blood or urine<sup>(7)</sup>.

Mild Acute pancreatitis: there is no multisystem failure and uncomplicated recovery.

Severe Acute pancreatitis: there is early or late local or systemic complications (multisystem failure) associated with high morbidity & mortality<sup>(7)</sup>.

Recurrent Acute pancreatitis: is defined as two or more attacks of pancreatitis associated with at least twice normal serum amylase levels<sup>(8)</sup>.

Chronic Pancreatitis: has been defined as a continuing inflammatory disease of the pancreas characterised by irreversible morphologic changes that typically causes pain and/or permanent loss of exocrine and/or endocrine function<sup>(7)</sup>.

### INCIDENCE:

Wang et al<sup>(9)</sup> from China showed that of 1471 cases of acute pancreatitis, only 157 cases (10.6%) were recurrent acute pancreatitis. Zhang et al<sup>(10)</sup> from Shanghai showed an incidence of 31.43% (77/245). The data from Europe by Gullo et al<sup>(11)</sup>



showed that out of the 1068 cases of acute pancreatitis ,288 (27%) had recurrence. Another study by Sanchez et al <sup>(12)</sup> in showed an incidence of 34.5%.

### **ETIOLOGY:**

Virtually any factor capable of causing an initial episode of acute pancreatitis has the potential to incite recurrent episodes <sup>(1)</sup>.

### **GALLSTONES :**

Only 3 to 7 percent of patients with gallstones develop pancreatitis<sup>(13,14)</sup>. Gender(risk greater in men. and stone size(stone diameter < 5 mm<sup>(15,16)</sup> may be risk factors. Cholecystectomy and clearing the common bile duct of stones prevents recurrence, confirming the cause-and-effect relationship<sup>(13)</sup>. The possible initiating event in gallstone pancreatitis may be reflux of bile into the pancreatic duct due to transient obstruction of the ampulla during passage of gallstones or obstruction at the ampulla secondary to stone(s) or edema resulting from the passage of a stone <sup>(17)</sup>. Serum alanine aminotransferase (ALT) concentration was the most clinically useful parameter in predicting a gallstone etiology in a meta-analysis<sup>(18)</sup>. ALT concentration of 150 IU/L or more (approximately a threefold elevation) had a positive predictive value of 95 percent for the diagnosis of gallstone pancreatitis.

### **BILIARY SLUDGE AND MICROLITHIASIS:**

Biliary sludge is a viscous suspension in gallbladder bile that may contain small stones (<5 mm in diameter), cholesterol monohydrate crystals or calcium bilirubinate granules which is formed by modification of hepatic bile by gallbladder mucosa<sup>(19-21)</sup>. Sludge is typically found in patients with functional or mechanical bile stasis due to prolonged fast, total parenteral nutrition, distal bile duct obstruction, rapid weight loss,

pregnancy, bone marrow or solid organ transplantation and drugs (ceftriaxone , cyclosporine and octreotide<sup>(22)</sup>) Sensitivity of USG abdomen for sludge is 60% while microscopic examination for cholesterol crystals has a sensitivity of 65-90% and EUS 95%. (19) Two uncontrolled studies suggest that biliary sludge can lead to pancreatitis as these patients benefit from intervention<sup>(21,23)</sup>.

Dissolution of microliths by UDCA , endoscopic sphincterotomy or cholecystectomy prevented further attacks of pancreatitis.

### **ALCOHOL:**

Approximately 10 percent of alcoholics develop attacks of acute pancreatitis even though the classic teaching is that alcohol causes chronic pancreatitis<sup>(24)</sup>. Some have nonprogressive recurrent alcohol-induced acute pancreatitis<sup>(25)</sup>.

Alcohol may act by inducing pancreatic acinar cells to synthesize the digestive and lysosomal enzymes responsible for acute pancreatitis or sensitize the acini to cholecystokinin<sup>(26)</sup>. The reason why only a small proportion of all alcoholics develop pancreatitis as well as the genetic and environmental influence in the development of pancreatitis in alcoholics is not clear.

### **SPHINCTER OF ODDI DYSFUNCTION:**

Sphincter of Oddi is a 5-15 mm long fibromuscular sheath surrounding the terminal CBD , pancreatic duct and common channel<sup>(27)</sup> Frequency of SOD in IRAP is 35%.(mean of 6 studies.) The terms papillary stenosis, sclerosing papillitis, biliary spasm, biliary dyskinesia, and postcholecystectomy syndrome have been used synonymously with sphincter of Oddi dysfunction (SOD).<sup>(28)</sup> . Two separate pathologic entities are described (1) Sphincter of Oddi stenosis (2) sphincter of Oddi dyskinesia

SOD has been associated with two clinical syndromes: biliary pain without other apparent causes in the setting of cholecystectomy (postcholecystectomy syndrome) and idiopathic recurrent pancreatitis and pancreatitis occurring after ERCP. <sup>(29,30)</sup>.

Gold standard for diagnosis is sphincter of Oddi manometry (SOM). <sup>(31)</sup>.

SOD in the setting of IAP is treated by endoscopic biliary or both biliary and pancreatic sphincterotomy. <sup>(32)</sup> Efficacy was 60-100% ,and complications were around 30 %.

Recurrence rate of SOD was about 10-20%. <sup>(32)</sup>

### **PANCREAS DIVISUM:**

It is the most common congenital pancreatic anomaly, occurring in approximately 7% of subjects in autopsy series (range 1 to 14 percent). It arises from failure of fusion of the ventral and dorsal pancreatic ducts in the second month of gestation, causing 80-95% of pancreatic juice to flow via dorsal duct through the minor papilla. The classic pancreas divisum consists of a small ventral duct which drains through the major papilla and the larger dorsal duct which drains through the minor papilla. In incomplete pancreas divisum, seen in 15 % of cases of pancreas divisum, a tiny branch of the ventral duct communicates with the dorsal duct. (15 percent ) <sup>(33)</sup>. In "reverse" divisum ,there is an isolated small segment of dorsal pancreas when the accessory duct of Santorini does not connect with the genu of the main pancreatic duct. Santorinicele refers to a cystic dilatation of the dorsal pancreatic duct in pancreas divisum as it enters the duodenal wall <sup>(34,35)</sup>. Although controversial, most believe that pancreas divisum is a cause of RAP occurring in about 20% of the patients <sup>(36)</sup> Diagnosis is by ERCP / MRCP <sup>(37)</sup>. Secretin improves ductal visualization during MRCP. Treatment is directed towards relieving outflow obstruction at the level of minor

papilla.Minor papillary sphincterotomy leads to symptom relief in 70-90%.

#### DRUGS :

Clear evidence of a definite association with pancreatitis exists for didanosine,sodium valproate , aminosaliclates, estrogen, calcium, anticholinesterases and sodium stibogluconate. An association likely but not definitely proven is for thiazide diuretics, pentamidine, ACE inhibitors, asparaginase, vinca alkaloids, some nonsteroidal anti-inflammatory drugs and clozapine. Pancreatitis is possibly caused by azathioprine, furosemide , tetracycline, metronidazole, isoniazid, rifampicin , sulphonamides, cyclosporin and some antineoplastic drugs.<sup>(38)</sup> The pathogenesis may be due to an idiosyncratic response in ( 6-mercaptopurine, aminosaliclates, sulfonamides) or to a direct toxic effect (eg, diuretics, sulfonamides)and angioedema of the gland with angiotensin converting enzyme inhibitors .

#### **HYPERTRIGLYCERIDEMIA:**

This accounts for 1.3 to 3.8 percent of acute pancreatitis<sup>(39)</sup>. Serum triglyceride concentrations above 1000 mg/dL can precipitate recurrent attacks of acute pancreatitis<sup>(40)</sup>. Another study by Fortson et al reported the cut off of 500mg/dL<sup>(39)</sup>. The pathogenesis of inflammation in this setting is unclear.This has been best studied in children with inherited disorders of lipoprotein metabolism associated with severe hypertriglyceridemia .The acquired causes of hypertriglyceridemia are alcohol abuse , obesity, diabetes mellitus, hypothyroidism, pregnancy, estrogen or tamoxifen therapy, glucocorticoid excess, nephrotic syndrome, and beta blockers <sup>(40)</sup>. The serum amylase may not be substantially elevated due to unclear reasons.

#### **HYPERCALCEMIA:**

Hypercalcemia of any cause can lead to recurrent acute pancreatitis<sup>(41)</sup>. The proposed mechanism is calcium activation of trypsinogen within the pancreatic parenchyma<sup>(42)</sup>. Even though, hyperparathyroidism is associated with pancreatitis, the actual incidence of recurrent acute pancreatitis in patients with hyperparathyroidism is low<sup>(43)</sup>

### **INFECTION:**

Infections associated with acute pancreatitis are<sup>(44,45)</sup>

Viruses — Mumps, Coxsackievirus, hepatitis A,B, and C , CMV, varicella-zoster, HIV

Bacteria — Campylobacter jejuni, Mycobacterium tuberculosis and avium complex,

Mycoplasma, Legionella, Leptospira, Salmonella

Fungi — Aspergillus

Parasites — Ascaris, Toxoplasma, Cryptosporidium, Microsporidium, Clonorchis .

### **BILIARY ASCARIASIS:**

Commonly reported from endemic regions of Far East, India, Latin America, and Middle East. Common disease presentations include biliary colic, obstructive jaundice, acalculous cholecystitis, choledocholithiasis, pancreatitis, and cholangitis.<sup>(46)</sup>,

In a study by Khuroo et al<sup>(46)</sup>, Ascariasis was the etiologic factor in 36.7% cases of pancreatitis. ERCP is used for diagnosis and extraction of worms . 90% patients recover with endoscopic treatment followed by anthelmintic therapy. Surgery is reserved for trapped worms in the ducts not removable by endoscopy.

### **TRAUMA:**

Blunt or penetrating trauma can damage the pancreas<sup>(47)</sup>. Healing of pancreatic ductal injuries leads to scarring and stricture of main pancreatic duct and pancreatitis.

## **VASCULAR DISEASES:**

Pancreatic ischemia from vasculitis (systemic lupus erythematosus and polyarteritis nodosa) <sup>(48)</sup> is a rare cause of significant recurrent pancreatitis. Most have mild attacks of pancreatitis secondary to ischemia.

## **TUMORS:**

5-7 % of patients with pancreatobiliary tumors present with RAP <sup>(27)</sup>. Pancreatic cancer should be suspected in any patient older than 40 or 45 years who has unexplained pancreatitis with a prolonged or recurrent course <sup>(49)</sup>. The tumors of the major duodenal papilla (adenoma and carcinoma and rarely islet cell tumors, lymphoma, neurofibroma, or metastatic tumors) can present with recurrent acute pancreatitis <sup>(50)</sup>.

Cystic pancreatic tumors <sup>(27)</sup> such as serous and mucinous cystadenomas and Intraductal papillary mucinous neoplasms (IPMN) can present with RAP. The pathognomonic duodenoscopic findings are a patulous papilla and mucin extrusion from the papillary orifice. They have an obvious malignant potential and early recognition and resection are the cornerstone of treatment. Ampullary tumors, the most common being adenomas are premalignant and have a more favourable prognosis with pancreatoduodenectomy. <sup>(49)</sup>

Endoscopic techniques can be used for small lesions which are benign or with carcinoma in situ, especially with ampullary adenomas. This can be achieved by snare ampullectomy or piecemeal snare resection. Tumor ablation can be done by NDYag laser, multipolar cautery, or argon plasma coagulation. Levy et al recommend an initial follow up after 3 months and yearly thereafter.

## **ANOMALOUS UNION OF PANCREATICOBILIARY DUCT.(AUPBD).**

This is defined as anomalous junction of the bile duct and pancreatic duct at an abnormally proximal site and long (>15mm )common pancreatobiliary channel.<sup>(27)</sup> The junction is located outside the duodenal musculature and not controlled by the sphincter of Oddi leading to regurgitation of pancreatic juice into biliary tract and vice versa causing recurrent acute pancreatitis.<sup>(51)</sup> The stasis of pancreatic secretion in the common channel as well as concurrent SOD can also cause pancreatitis. AUPBD is frequently associated with choledochal cyst and is more common in East Asia<sup>(52)</sup> The diagnosis is made on ERCP ; the pancreatic duct and bile duct fills simultaneously with contrast and connect with an obviously long channel(>15mm in adult) <sup>(53,54)</sup> When the amylase in the aspirated bile is high,the presence of AUPBD is strongly suggested.<sup>(55)</sup> Endoscopic sphincterotomy may be effective in preventing recurrent attacks.<sup>(55)</sup> AUPBD is a premalignant condition that can progress to gall bladder or cholangiocarcinoma .Prophylactic cholecystectomy is recommended in patients with AUPBD with no biliary dilatation.<sup>(56)</sup> But in patients with AUPBD and choledochal cyst, extensive resection of extrahepaic bile duct along with cyst and biliary diversion procedure is recommended.

## **CHOLEDOCHOCELE:**

This is a prolapse or herniation of the intramural segment of the distal CBD into the duodenal lumen. Todani classified it as Type III choledochal cyst. <sup>(57)</sup> Recurrent pancreatitis occurs in 12-25 % of patients<sup>(58)</sup> .Choledochoceles may cause intermittent obstruction of the pancreatic duct when it become distended with bile.Reflux of bile into

the pancreas can activate pancreatic enzymes leading to recurrent episodes of pancreatitis<sup>(58,59)</sup>. Diagnosis is by ERCP<sup>(60)</sup>. Endoscopically, the papilla has a bulging appearance and feels soft (Pillow sign) when pressure is applied with the catheter tip<sup>(61)</sup>. Treatment includes endoscopic sphincterotomy and unroofing of the choledochocoele with a papillotome<sup>(62)</sup>. Surgical excision is done for a large choledochocoele .

### **ANNULAR PANCREAS:**

This is a congenital anomaly that manifests as a band of pancreatic tissue partially or completely encircling the duodenum ,usually at the level of or just proximal to the major duodenal papilla<sup>(63)</sup>. Symptoms begin in childhood with duodenal obstruction. Childhood disease is associated with congenital anomalies such as Downs syndrome, cardiac defects tracheoesophageal fistula, Meckels diverticulum and imperforate anus<sup>(64)</sup>. Adults may present with recurrent acute pancreatitis, chronic pancreatitis, peptic ulcer or biliary obstruction<sup>(2)</sup>. Pancreatitis may be due to stasis of pancreatic juice<sup>(63)</sup>. Pancreas divisum is present in one third of patients .<sup>(65)</sup> Diagnosis is by ERCP<sup>(63)</sup>. Gastrojejunostomy may be required to bypass the obstructed bowel segment<sup>(66)</sup>. Division of annulus ,once the procedure of choice ,is no longer performed because of the risk of pancreatitis and pancreatic fistula.

### **GENETIC MUTATIONS:**

#### **TRYPSIN AND ACUTE PANCREATITIS**

Acute pancreatitis is caused by unregulated trypsin activity within the pancreatic acinar cell or pancreatic duct that leads to zymogen activation, pancreatic autodigestion, and pancreatic inflammation ie pancreatitis<sup>(67)</sup>



Trypsin:- is a major pancreatic serine protease with two globular protein domains connected by a single side chain on the surface opposite the active site. Trypsinogen becomes trypsin with cleavage of a short exposed peptide chain called trypsinogen activation peptide by the action of enterokinase or by a second trypsin molecule. The trypsin contains a calcium binding pocket near the side chain connecting the two globular domains. This side chain (autolysis loop) contains an arginine residue at amino acid position 117 (coded for by codon 122 — ie R122), which is a target for attack by other trypsin enzymes. Enzymatic cleavage of the side chain at R122 by the second trypsin leads to rapid destruction of the first trypsin molecule (autolysis). As the concentration of soluble calcium rises, calcium enters the calcium binding pocket and limits exposure of R122 to enzymatic attack by another trypsin<sup>(68)</sup> and protects trypsin from autolysis.

Trypsin is susceptible to rapid autolysis within the acinar cell where calcium levels are low, protected from autolysis after active secretion into the pancreatic duct and duodenum where calcium levels are high, and then undergoes autolysis in the distal small intestine after calcium is absorbed in the distal duodenum and jejunum.

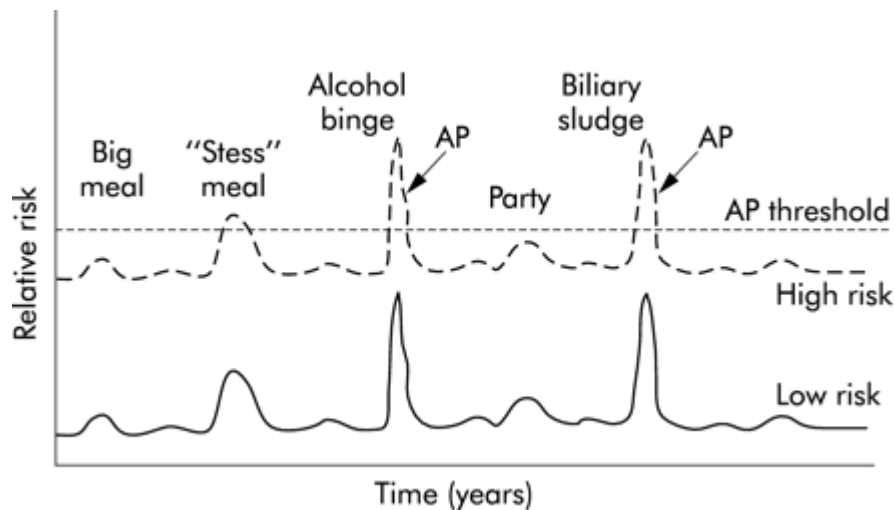
Protective mechanisms against acute pancreatitis:

Intracellular protective mechanisms include synthesis of trypsin as an inactive zymogen (trypsinogen), zymogen compartmentalisation and packaging, synthesis of a specific trypsin inhibitor ( PSTI or SPINK1), R122 dependent trypsinogen / trypsin autolysis, control of intra-acinar cell calcium levels (to facilitate autolysis), and lysosome dependent pathways of zymogen/activated digestive enzyme elimination<sup>(69)</sup>. Once the zymogens are secreted into a calcium rich juice (eliminates the autolysis protective

mechanism) the pancreas is dependent on SPINK1 (inhibit prematurely activated trypsinogen), and rapid flushing of the pancreatic duct by fluid from the duct cells to protect against pancreatitis. Duct flushing is a cystic fibrosis transmembrane conductance regulator (CFTR) dependent protective mechanism. Disruption of any of these protective mechanisms increases susceptibility to acute pancreatitis and predisposes to chronic pancreatitis. The combined effect of environmental and genetic factors in increasing the risk of recurrent acute pancreatitis is given in figure below.

Relationship between risk factors and genetics in developing recurrent acute

Pancreatitis<sup>(69)</sup>:-



It is possible that environmental triggering factors leads to acute pancreatitis in a susceptible individual with underlying genetic predisposition<sup>(69)</sup>

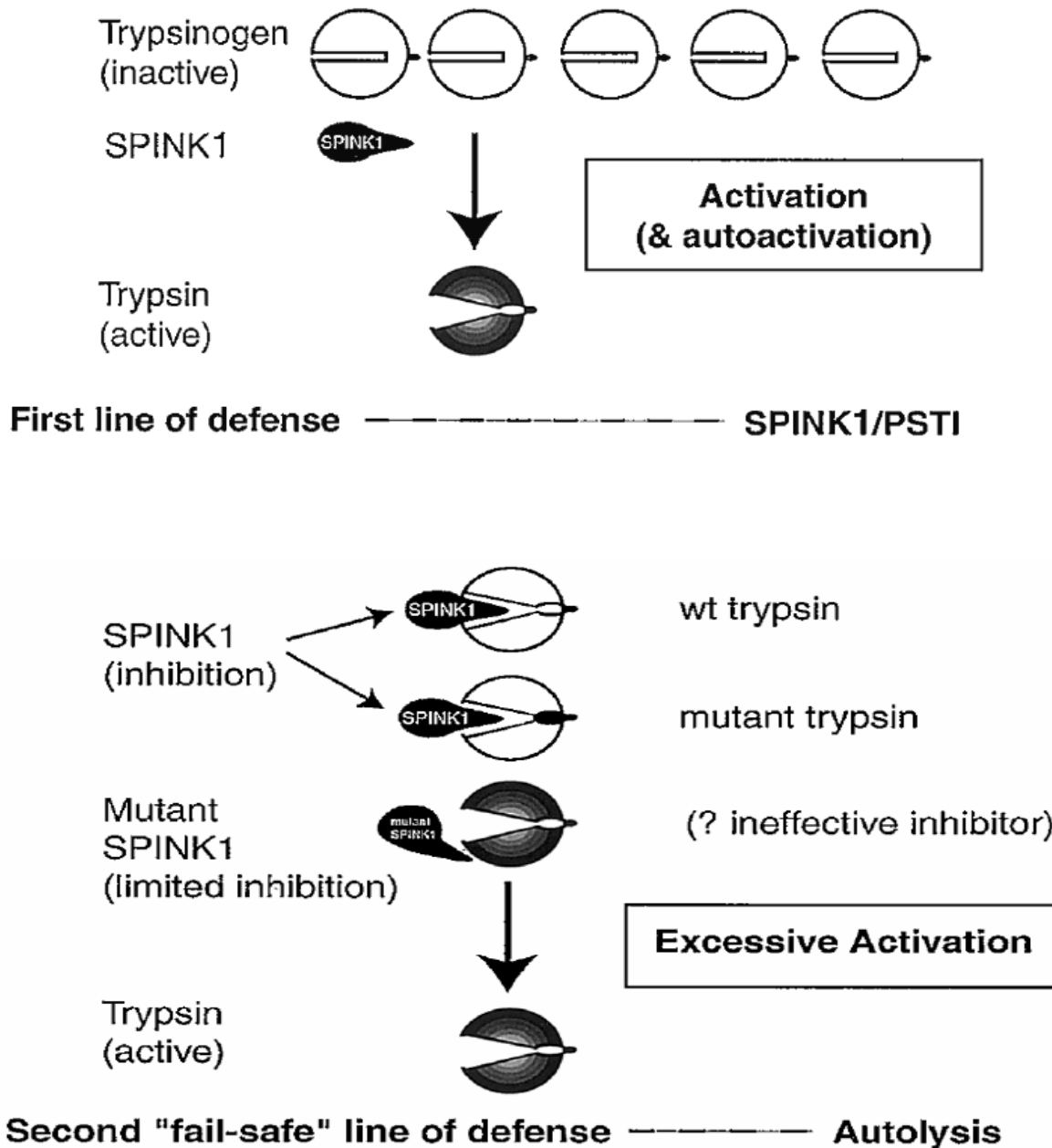
Cationic Trypsinogen mutations:

Most cases of hereditary pancreatitis are associated with mutations in the cationic trypsinogen gene (PRSS1)<sup>(4)</sup>. The R122H and N29I mutations which are gain of function mutations interferes with autolysis and/or cause premature trypsinogen activation.<sup>(70)</sup> Between 60% and 80% of individuals who inherit the mutation

usually develop pancreatitis<sup>(69)</sup> Approximately half of individuals with acute pancreatitis will develop chronic pancreatitis, and up to 40% of individuals with chronic pancreatitis will develop pancreatic cancer. A patient with a gain of function PRSS1 gene will have at least one other family member with recurrent acute or chronic pancreatitis<sup>(71)</sup>

## SPINK1 mutations:

### Intrapancreatic Zymogens



SPINK1 acts as the first line of defense against prematurely activated trypsinogen in acinar cells. If there is ineffective inhibition by mutant SPINK1, free trypsin activity increases and initiates activation of other zymogens. SPINK1 N34S allele is associated

with acute and chronic pancreatitis<sup>(72)</sup>. The association is weak, with <1% of mutation carriers developing pancreatitis sometime during their lifetime<sup>(69)</sup>. The SPINK1 N34S allele acts as either a modifier gene or a susceptibility factor for a polygenic complex trait.<sup>(73)</sup> Felderbauer and colleagues identified an interaction between SPINK1 and mutations in the calcium sensing receptor gene. These mutations cause familial hypocalciuric hypercalcaemia, characterised by benign elevation in plasma calcium levels.<sup>(74)</sup> Patients with both HFF and the SPINK1 N34S allele mutations have multiple episodes of recurrent abdominal pain, pancreatitis, and eventually developed chronic pancreatitis. The SPINK1 N34S allele modifies the HFF phenotype to cause recurrent acute and chronic pancreatitis<sup>(75)</sup>

#### CFTR gene mutations

The CFTR functions as a chloride channel in the apical membrane of most secretory epithelium including pancreatic ductal epithelial cells. With CFTR gene mutations, ductal water flow is reduced owing to defective anion secretion. Protein concentration in the duct rises which causes precipitation of protein and plugging of ductal lumina<sup>(69)</sup>.

CFTR gene is located on the long arm of chromosome 7 (7q31). The most common mutation causing cystic fibrosis is a 3- base pair deletion causing loss of phenyl alanine 508( Delta F 508). When both alleles of CFTR gene are affected by severe mutations, classic cystic fibrosis results with pancreatic insufficiency. Compound heterozygosity of CFTR gene with one severely affected and one moderately affected allele has been shown to be a cause of recurrent pancreatitis. In these patients, there are

no clinical signs of classic cystic fibrosis , no nasal polyps and sweat chloride is within the normal range<sup>(69)</sup>

CFTR may be part of a complex in which heterozygous CFTR and heterozygous SPINK1 mutations cause recurrent acute pancreatitis when occurring together. CFTR variants may also increase susceptibility to environmental factors such as alcohol<sup>(69)</sup>

### Cathepsin B (CTSB)

Cathepsin B is a lysosomal cysteine proteinase , involved in the initial activation of trypsinogen .CTSB is abundantly secreted from the human exocrine pancreas and contained in the pancreatic secretory zymogen granules rather than in the lysosomes CTSB can activate trypsinogen in vitro. In fact 90% of the activation of trypsinogen during experimental pancreatitis is accounted for by CTSB and it disappears when the *ctsb*- gene is deleted<sup>(76)</sup>

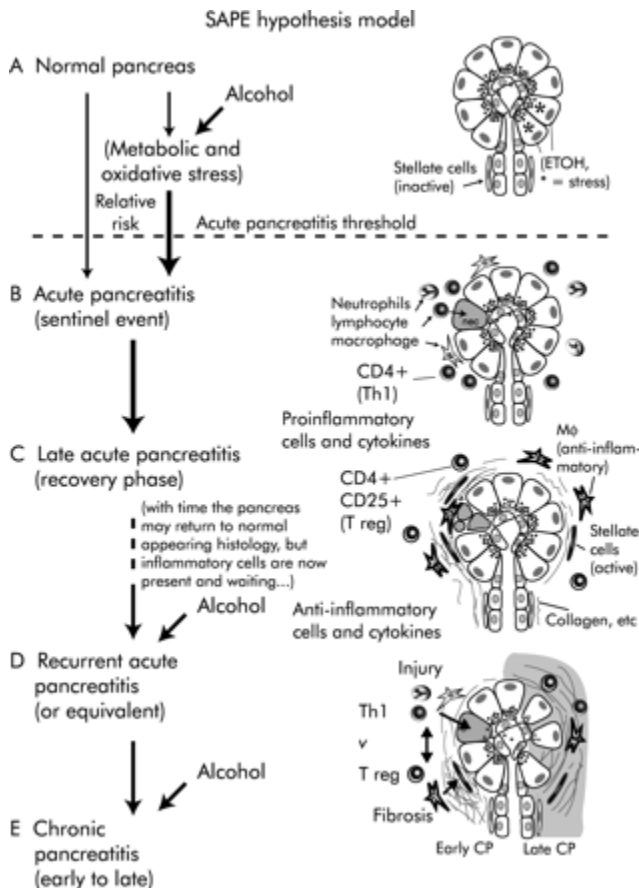
A recent study by Madhurkar et al compared 2 separate cohort of patients with tropical pancreatitis totalling 306 patients (about 50% had SPINK1 mutations) with 330 controls. C76G polymorphism that results in leucine to valine mutation at aminoacid 26 was twice as common in patients with tropical pancreatitis. CTSB mutation also doubled the risk for tropical pancreatitis irrespective of whether patients were positive or negative for N34S mutation in SPINK1<sup>(77)</sup>

Recurrent acute pancreatitis has rarely been described in patients with suprapapillary duodenal diverticulum,brunneromas and hydatid cyst of the head of pancreas.

## **PATHOGENESIS:**

Though a number of animal models have been developed ,none is strictly compared to the human condition.

SAPE (sentinel acute pancreatitis event) hypothesis model <sup>(78)</sup> :-



Precipitants like alcohol ,genetic and other environmental factors reduces the threshold for acute pancreatitis and the first or sentinel acute pancreatitis event initiates the inflammatory process with infiltration of the gland by inflammatory cells.

In late acute pancreatitis ,anti-inflammatory cells limits inflammation and healing starts with activation of stellate cells and consequent fibrosis. Recurrent episodes perpetuates these events and lead to acinar cellloss and sclerosis leading to chronicity.

## **EARLY ACUTE CHANGES:**

### Intraacinar activation of proteolytic enzymes:

The intrapancreatic release of trypsin leads to further activation of more trypsin, and other enzymes like phospholipase, chymotrypsin, elastase, activation of complement, kallikrein-kinin, coagulation, and fibrinolysis. The active enzymes leads to pancreatic autodigestion, and spread of destruction into the peripancreatic tissue<sup>(79)</sup>

### Microcirculatory injury:

The enzymes damage the vascular endothelium , interstitium and the acinar cells<sup>(79)</sup> leading to vasoconstriction, capillary stasis, decreased oxygen saturation, and progressive ischemia, These changes lead to increased vascular permeability and swelling of the gland (edematous or interstitial pancreatitis)<sup>(80)</sup>. Reperfusion of damaged tissues leads to the release of free radicals and inflammatory cytokines into the circulation, causing further injury<sup>(80)</sup>

Leukocyte chemoattraction, release of cytokines, and oxidative stress. There is glandular invasion by macrophages and polymorphonuclear leukocytes in early stages<sup>(81)</sup> with subsequent release of proinflammatory cytokines (TNF, IL-1, 6, and 8), arachidonic acid metabolites (PG , PAF, and LT ), proteolytic and lipolytic enzymes, and reactive oxygen metabolites .These substances also interact with the pancreatic microcirculation to increase vascular permeability and induce thrombosis and hemorrhage, leading to pancreatic necrosis.

### Systemic Response:

Some patients develop systemic inflammatory response and present with fever, acute respiratory distress syndrome (ARDS), pleural effusions, renal failure, shock, and



myocardial depression. Metabolic complications include hypocalcemia, hyperlipidemia, hyperglycemia, hypoglycemia, and diabetic ketoacidosis.<sup>(82)</sup>

Bacterial translocation:

The human gut barrier is compromised due to ischemia and pancreatitis-induced gut arteriovenous shunting, leading to translocation of bacteria resulting in local and systemic infection<sup>(83)</sup>. Local bacterial infection of pancreatic and peripancreatic tissues results in septic complications and multiorgan failure .

**CLINICAL FEATURES:**

Acute pancreatitis is an important cause of acute upper abdominal pain and its clinical features are similar to a number of other acute illnesses.<sup>(84)</sup>

Symptoms — Almost all patients have recurrent attacks of acute upper abdominal pain at the onset . The pain is steady and may be in the mid-epigastrium, right upper quadrant, diffuse, or, to the left side. Pain can last for days. Its onset is rapid, reaching maximum intensity in many cases within 10 to 20 minutes. In about one-half of patients, pancreatic pain characteristically has band-like radiation to the back. Acute pancreatitis related to alcohol frequently occurs one to three days after a binge or cessation of drinking. Painless disease is uncommon (5 to 10 percent) and seen in the postoperative setting (renal transplantation), peritoneal dialysis, Legionnaire's disease.

The abdominal pain is typically accompanied ( 90 percent ) by nausea and vomiting, which may persist for many hours. Restlessness, agitation, and relief on bending forward are other notable symptoms. Patients with fulminant attacks may present in shock or coma. Patients with recurrent episodes of pancreatitis sometimes manage their

flares at home by staying on clear liquids for a few days. Hemorrhagic complications are rare including ecchymotic discoloration of the flanks or hemosuccus pancreaticus .

#### Physical examination:

Physical findings vary depending upon the severity of an attack. Systemic features include fever, tachycardia, in severe cases, shock and coma. In mild disease, the epigastrium may be minimally tender. Severe episodes are often associated with abdominal distention, especially in the epigastrium, and tenderness and guarding.

Respiration may be shallow due to diaphragmatic irritation from inflammatory exudate, and dyspnea may occur due to associated pleural effusion. Ecchymotic discoloration in the flank (Grey-Turner's sign) or the periumbilical region (Cullen's sign) occurs in 1 percent of cases . Jaundice is usually the result of obstruction of the CBD. An epigastric mass due to pseudocyst formation may become palpable in the course of the disease.

Less common features include subcutaneous nodular fat necrosis panniculitis, thrombophlebitis in the legs, and polyarthrititis. The lesions of fat necrosis, 0.5 to 2 cm tender red nodules, are usually located over the distal extremities . There may also be findings indicative of underlying disorders such as hepatomegaly in alcoholic pancreatitis, xanthomas in hyperlipidemic pancreatitis, and parotid swelling associated with mumps.

#### **LABORATORY TESTS:**

Serum amylase— rises within 6 to 12 hours of onset, and is cleared fairly rapidly from the blood (half-life 10 hours). In uncomplicated attacks, serum amylase is usually elevated for three to five days except in hypertriglyceridemia-associated pancreatitis <sup>(39)</sup>.

The serum amylase concentration in acute pancreatitis is usually more than three times the upper limit of normal but it may be normal or minimally elevated.

Hyperamylasemia may also be seen in diseases of salivary glands and fallopian tubes, intestinal infarction and perforated viscus and peritonitis, renal failure, cholecystitis, macroamylasemia. Analysis of amylase of pancreatic origin (P-isoamylase) which represents 35 -50 % of the normal serum amylase improves diagnostic accuracy<sup>(85)</sup>

Serum lipase — Its sensitivity for the diagnosis of acute pancreatitis ranges from 85 to 100 percent in various reports<sup>(85)</sup>. Lipase is more specific than serum and urinary amylase in diagnostic accuracy both on day one and day three<sup>(86)</sup>. Nonspecific elevations of lipase have been reported in almost as many diseases as amylase<sup>(87)</sup>. Lipase elevations occur earlier and last longer than amylase elevations. Studies found that the combination of enzymes does not improve diagnostic accuracy<sup>(88)</sup>. Daily measurement of enzymes has no value in assessing the clinical progress or prognosis<sup>(87)</sup>. The level of pancreatic enzyme elevation does not correlate with severity

### **BILE MICROSCOPY:**

Microscopic examination of gall bladder bile is considered to be the diagnostic gold standard for detection of microlithiasis. Bile can be collected during ERCP or from the duodenum. However, the preferred method is collection of gall bladder bile by administering cholecystokinin<sup>(19)</sup>. The collected bile should be immediately centrifuged and analysed. Cholesterol microcrystals are birefringent and rhomboid shaped and best seen on polarizing microscopy while bilirubin granules are red brown and detected by conventional microscopes.

## **RADIOLOGIC FEATURES :**

Abdominal plain radiograph— excludes obstruction and bowel perforation. Radiographic findings are "sentinel loop" or the "colon cutoff sign" and generalized ileus .

Abdominal ultrasound —A diffusely enlarged, hypoechoic pancreas is the classic ultrasonographic image of acute pancreatitis. Ultrasound also detects gallstones and image the biliary tree. 25 to 35 percent have bowel gas that may obscure the pancreas. Ultrasound cannot clearly delineate extrapancreatic spread of pancreatic inflammation or identify necrosis within the pancreas. The accuracy of USG in detecting cholelithiasis is high and the sensitivity ranges from 92 to 96%.<sup>(89)</sup> When the stones are <3mm in diameter, the sensitivity of USG is only 55 to 65%.<sup>(90)</sup> The sensitivity of USG for dilated CBD is 55-90% and CBD stones is 50-60%.<sup>(89)</sup>

CT scan — the most useful imaging study for diagnosis of acute pancreatitis, intraabdominal complications and also for assessment of severity. Pancreatic necrosis is seen as unenhanced areas (less than 50 Hounsfield units after IV contrast) greater than 3 cm in size after oral and intravenous contrast. If there is suspicion of infection, analysis of contents obtained by CT or US guided needle aspiration can differentiate between sterile and infected pancreatic necrosis or a pseudocyst. CT abdomen useful to detect pancreas divisum and pancreatic tumors. But it is less accurate to identify gall stones<sup>(91)</sup>

Magnetic Resonance Imaging: The advantages of MRI over CT include lack of nephrotoxicity of gadolinium, ability of MRI to better categorize fluid collection as acute fluid collections, necrosis, abscess, hemorrhage and pseudocyst, and the greater

sensitivity of MRI to detect mild acute pancreatitis compared to CT. MRI was reliable for staging the severity of AP, had prognostic value, and was associated with fewer contraindications compared to CT, and was able to detect pancreatic duct disruptions. Abdominal imaging using MRI is operator dependent with consequent variability in quality and technique<sup>(92)</sup>.

Magnetic Resonance Cholangiopancreatography: permits detailed anatomic images of pancreatic and bile ducts and produce an image similar to ERCP. In RIAP, the primary value of MRCP is to identify congenital anomalies of pancreatobiliary ducts such as pancreas divisum, choledochocoele, AUPBD, and annular pancreas<sup>(92)</sup>

In the diagnosis of pancreas divisum, MRCP has been reported to be as good as or even superior to ERCP<sup>(93)</sup> MRCP is also helpful for diagnosing or excluding choledocholithiasis and is comparable to ERCP (Sensitivity - 90%).<sup>(94)</sup> However small gallstones (<5 mm) in the biliary tree are easily missed<sup>(95)</sup>

Recently, dynamic MRCP, acquisition of multiple pictures at short intervals after intravenous secretin enhances visualization of the pancreatic ducts.<sup>(96,97)</sup>

Costamagna et al.<sup>(35)</sup> used secretin-enhanced MRCP to document Santoriniceles in patients with idiopathic acute recurrent pancreatitis when compared with conventional MRCP.

Persistent dilatation of MPD with secretin-enhanced MRCP may identify functional outflow obstruction at major or minor duodenal papilla.<sup>(96)</sup>

In the near future, MRCP may replace diagnostic ERCP for the evaluation of ductal anatomy.<sup>(97)</sup> and reserve the use of ERCP for a therapeutic procedures .

The major advantage of MRCP is that it does not require endoscopy, contrast injection, or

radiation and is safe <sup>(98)</sup> in pregnancy and those with contrast allergy.

Endoscopic Retrograde Cholangiopancreatography:- can define anatomic or structural abnormalities by observing duodenal papilla and obtaining ductography.<sup>(99)</sup> and to collect bile for crystal analysis .The most frequently identified causes of unexplained pancreatitis after endoscopic evaluation are ampullary lesions, choledocholithiasis, gall bladder microlithiasis, pancreas divisum , AUPBD , annular pancreas, pancreatic cancer, SOD and chronic pancreatitis. When bile is collected from CBD at ERCP, obtaining gallbladder bile with cholecystokin stimulation is ideal.<sup>(19)</sup> Bile aspiration after deep cannulation of CBD without cholecystokin stimulation is more practical because it is simple and easy.

When bile collection, ductography, and sphincter of manometry (SOM) are performed in one session, studies are recommended to be carried out in the following order; bile collection, ductography, and lastly SOM.To summarise , ERCP plays an important role in the management of RIP. The diagnostic yield of ERCP varies from 38% to to 79% .

Endoscopic Ultrasonogram: is an ideal minimally invasive tool for evaluation of unexplained pancreatitis. It may be used for identification of cholelithiasis , gallbladder sludge, CBD stones,<sup>(100)</sup> pancreatic tumors, chronic pancreatitis and ampullary lesions.

### APPROACH TO DETERMINE ETIOLOGY OF RECURRENT PANCREATITIS:-

Evaluation of a patient after the first attack of pancreatitis includes a careful history , lab studies ( lipid profile ,serum calcium, ANA, LFT) and USG abdomen. An USG should be repeated because multiple longitudinal examinations may be required to identify small gallbladder stones or sludge.<sup>(102,103)</sup> Approximately 20% to 50% of patients with acute pancreatitis will have a recurrence.<sup>(11,27,105,106)</sup> When a patient has a recurrent attack, invasive imaging studies should be performed because a diagnosis can be established in 38% to 76%.<sup>(101,105,107,108)</sup>

EUS if available should be the next test as it can identify gall bladder sludge, small CBD stones , pancreas divisum , pancreatic tumors and early chronic pancreatitis.If EUS is normal or not available , ERCP with SOM is recommended.

In clinical practice, ERCP without SOM is commonly performed presumptively to exclude CBD stones, yet their prevalence is low in RIP (<10%) and much less frequent than SOD. Because of the substantial risk of pancreatitis after ERCP in patients with SOD, such a practice is discouraged unless clinical, laboratory<sup>(103,104,109)</sup> and radiologic features strongly suggest choledocholithiasis as a likely cause.

For patients with idiopathic acute pancreatitis, selective use of genetic testing may be appropriate. For the patient with RIAP, especially in the setting of an appropriate family history, genetic testing for cationic trypsinogen gene (PRSS1) is recommended<sup>(92)</sup> Other genetic abnormalities associated with idiopathic pancreatitis include mutation of cystic fibrosis transmembrane conductance regulator gene (CFTR) and SPINK1 gene. The role of genetic testing for these mutations is less clear and controversial<sup>(70)</sup>.

## **TREATMENT:**

### **GENERAL PRINCIPLES OF THERAPY:**

Mild pancreatitis is treated with supportive care including pain control, intravenous fluids, and nothing by mouth. In severe pancreatitis, intensive care unit monitoring and support of pulmonary, renal, circulatory and hepatobiliary function may minimize systemic sequelae. Fluid resuscitation is particularly important and around 250 to 300 cc of intravenous fluids per hour are required for 48 hours. <sup>(110)</sup>

Preventing infection in severe acute pancreatitis : Three approaches have been taken to decrease bacterial infections in acute necrotizing pancreatitis : Enteral feeding , selective decontamination of the gut with non-absorbable antibiotics and prophylactic systemic antibiotics. Guidelines from the American Gastroenterological Association (AGA) <sup>(110)</sup> recommended antibiotics for patients with >30 percent pancreatic necrosis. Due to the conflicting data, the use of prophylactic imipenem/meropenem in severe acute pancreatitis, with significant necrosis and/or organ failure, continues to be debated.

Nutrition: Nutritional support is required for patients with severe pancreatitis. Parenteral feeding is recommended if there is ileus. Enteral feeding should be started to reduce systemic complications. Three meta-analysis of enteral nutrition suggest improved outcomes compared with parenteral nutrition <sup>(111)</sup>. Human studies have shown that continuous feeding in the distal jejunum does not stimulate exocrine pancreatic secretion <sup>(112)</sup>

Open surgical debridement:- is recommended for patients with infected necrosis and those deteriorating with conservative management. <sup>(84)</sup>.



Experimental agents:—Data suggests that there is no role for proteinase inhibitor gabexate mesilate, somatostatin and its analogue octreotide<sup>(113,114,115)</sup> and platelet activating factor antagonist, lexipafant in therapy of RAP.<sup>(116)</sup> Correcting underlying predisposing factors of pancreatitis is very important to prevent recurrence.

## METHODOLOGY

### **A. RETROSPECTIVE ANALYSIS OF PATIENTS WITH RECURRENT ACUTE PANCREATITIS (RAP):**

#### **STUDY DESIGN:**

Retrospective descriptive study.

Retrospective analysis of patients diagnosed to have recurrent acute pancreatitis attending Christian Medical College Hospital, Vellore during the period January 2002 to December 2007 (5 years).

#### **SUBJECTS:**

188 patients with two or more episodes of acute pancreatitis were included in the analysis.

#### **DEFINITIONS:**

Diagnostic criteria for recurrent acute pancreatitis<sup>(8)</sup>

- ( i ) Two or more documented episodes of typical pancreatic type of abdominal pain more than two months apart.
- (ii ) Amylase or lipase greater than 3 times the upper limit of normal and / or
- (iii ) Features of acute pancreatitis on imaging studies ( Ultrasound / CT abdomen )

Criteria for Severe Acute Pancreatitis<sup>(7)</sup>– Acute Pancreatitis with one or more of the following:

Local complications: pancreatic necrosis, abscess or pseudocyst,

Systemic organ dysfunction: respiratory failure , renal failure, shock .

Biliary pancreatitis: Pancreatitis with one or more of the following: ( i )Clinical jaundice or elevated serum bilirubin ( direct bilirubin>50% of total bilirubin), (ii) elevated ALT, AST, ALP,(iii) dilatation of bile duct , gallstone or sludge in the gall bladder , common bile duct stone by ultrasound /CT abdomen / ERCP / MRCP imaging (iv) microlithiasis on bile duct / duodenal bile sampling .

Alcohol induced pancreatitis: Pancreatitis in a person consuming an average of 80 g alcohol taken daily for more than 5 years or an alcoholic binge taken immediately before the acute attack.

Hyperlipidemia induced pancreatitis: Pancreatitis with serum triglyceride more than 500mg /dL).

Hypercalcemia induced pancreatitis: Pancreatitis with fasting serum calcium equal to or more than 10.5 mg /dL (hospital reference values).

Idiopathic pancreatitis: No etiology detected on history, clinical examination , laboratory tests , radiological tests,bile sampling or ERCP .

#### **EXCLUSION CRITERIA:**

All patients diagnosed to have chronic pancreatitis on ultrasound / CT abdomen (intraductal or parenchymal calcification or dilatation of pancreatic duct , atrophy of pancreas ) or ERCP (abnormal main pancreatic duct or > 3 abnormal side branches) were excluded.

#### **ANALYSIS:**

Clinical profile, etiology, complications and response to therapy were analysed.

## **B. GENETIC STUDIES IN RECURRENT ACUTE PANCREATITIS (RAP):**

Seventeen patients with recurrent acute pancreatitis were prospectively analysed for known genetic mutations or polymorphisms in PRSS1, PRSS2, SPINK1 and Cathepsin B genes.

### **GENETIC ANALYSIS:**

#### DNA extraction using manual method for RAP:

10 ml of peripheral blood was collected in an EDTA tube and centrifuged at 4000 rpm for 10 minutes at 4°C. The buffy coat was transferred to a fresh tube to which 10-15 ml of RBC lysis solution was added and incubated in ice for 10 minutes (a). The mixture was centrifuged at 4000 rpm for 10 minutes at 4°C and the supernatant discarded (b). The steps (a) and (b) are repeated to obtain a clear white pellet. 4.5 ml WBC lysis solution, 250µl of 10% SDS and 100 µl of 10mg/ml proteinase K are added to this pellet and incubated at 37°C overnight. After the lysis is complete, 1.5ml 6M NaCl is added along with an equal volume of chloroform and centrifuged at 4000 rpm for 30 minutes for 4°C. The upper layer is taken and suspended in 5ml saturated phenol and shaken well. This is centrifuged at 4000 rpm for 30 minutes at 4°C. The supernatant is taken and suspended in chloroform : Octanol (24:1) and centrifuged at 4000 rpm for 30 minutes at 4°C. To the supernatant, double the volume of absolute alcohol is added in a separate tube and then the supernatant is suspended in alcohol. This is mixed gently and the pellet is transferred into 70% ethanol in a micro centrifuge tube. After the pellet is transferred into 100-300 µl 1XTE buffer with a pH of 7.4 and stored at 4°C.

#### DNA extraction using isolation kit for control samples:

Blood samples were collected in EDTA tubes from peripheral vein and stored at – 20 °C. DNA was isolated from this whole blood using the Pure Gene DNA isolation kit according to the instructions from the manufacturer (Gentra Systems, Minneapolis, Minnesota, USA). DNA was diluted to a concentration of 50 milligram per millilitre and stored at 4 °C.

#### PCR- Polymerase Chain Reaction:

PCR was performed in 25 mL with 250 ng of genomic DNA, a mixture containing 50 mmol/L Tris.HCl, pH 9.2, 2.25 mmol/L magnesium chloride (MgCl<sub>2</sub>), 7.5% DMSO, 16 mmol/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 250 mmol/L each of dGTP and deazadGTP, 500 mmol/L of the other dNTPs, and 3.3 U of Expand Long Template DNA polymerases (Roche Diagnostics, GmbH, Mannheim, Germany). After 5 minutes of denaturation at 94°C, 30 cycles were performed at 94°C for 40 seconds, an annealing temperature (Table A) for 30 seconds , and 72°C for 30 seconds for 30 cycles, with the final extension of 72°C for 7 minutes and the programme ends. The Oligonucleotides used are given in the table below. Table A

Mutation	Oligonucleotides	Ann. temp
N34S	5'TTCTGTTTAATTCCATTTTTAGGCCAAATGCTGCA 3' 5'GGCTTTTATCATACAAGTGACTTCT3'	56
N29I	5'CCATCTTACCCAACCTCAGTAG3' 5'TGATGACAGATCGTTGGGGGCTAGA 3	56
R122H	5'GGTCCTGGGTCTCATACCTT3' 5'GTAATGGGCACTCGAAATGT3'	52

#### Conformation sensitive gel electrophoresis (CSGE):

For control analysis, 3µl of heteroduplexed control samples were mixed with 1.5µl of loading buffer (70% glycerol, 0.1% Xylene cyanol, 0.1% Bromophenol blue, 0.01% 1M EDTA) and electrophoresed in a mildly denaturing gel (400x330x1mm in size) containing 10% Acrylamide {99.1 Acrylamide (Invitrogen Lifetechnologies, Groningen, Netherlands): Bisacryloylpiperazine (Fluka Chemie, Buchs, Switzerland)}, 10% ethylene glycol (Sigma, St.Louis, USA), 15% Formamide (Sigma, St.Louis, USA) and 0.5X TTE buffer [20X TTE -1.78 M Tris (USB, Ohio, USA), 570mM Taurine (USB, Ohio, USA) and 4mM EDTA (USB,Ohio,USA)]. Polymerization was achieved by the addition of 0.1% Ammonium persulphate (USB, Ohio, USA), and 0.07% N, N, N', N' – Tetramethylethylenediamine (TEMED) (Sigma, St.Louis, USA). Following electrophoresis at 400V for 18 hours, bands were visualized by ethidium bromide staining.

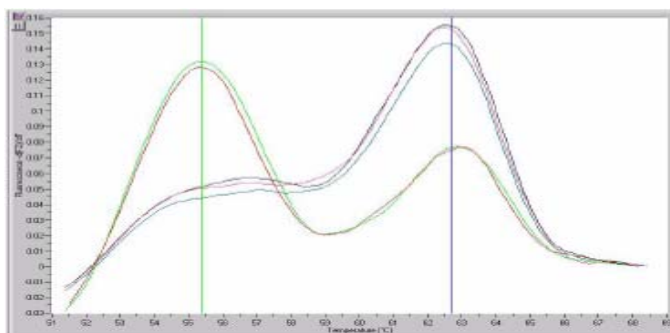
We employed two different assays for detection of the PRSS2 G191R allele.

Method A. Real time detection: A dual-colour allele-specific discrimination assay for genotyping the exon 4 mutation at codon 191 of the *PRSS2* gene was using the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories) with molecular beacons. PCR was performed with the forward primer 5'-CTA TTG TCT CTT TCT CTG GCC TA-3' and the reverse primer 5'-GCC ACC TTG GGA GTT CAA ATC A-3' in the presence of the developed molecular beacons, FAM- labeled wild-type beacon (5'-CGC GTC ATC CTT GCC TCC CTC GAG GAA GCC GAC GCG-3') and the HEX-labeled mutant beacon (5'- CGC GTC ATC CTT GCC TCT CTC GAG GAA GCC GAC GCG-3'). The 25 microliter reaction mixture contained 200 ng of genomic

DNA, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 4 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, 5 pmol of each primer, 200 nM of each beacon and 2.5 U Taq-DNA-polymerase. The PCR conditions were 3 min at 95°C, then 40 cycles of 30 s at 95°C, 30 s at 59°C and 30 s at 72°C. Fluorescent signals were measured at 59°C (Figure III). Genotypes were assigned using the iCycler iQ Optical System Software version 3.1.

**Method B. Melting curve analysis:** We performed melting curve analysis for the G191R alteration using a pair of fluorescence resonance energy transfer (FRET) probes and the LightCycler (Roche Diagnostics). Primer for PCR of exon 4 are described above. The donor probe was 5'-CAGGAATCCTTGCCTCCCTC-FL (FL: 5,6-carboxyfluorescein attached to 3'-O-ribose) and the acceptor probe was 5'-LC-AGGAAGCCCACACAGAACATGTTGTTG-ph (LC: LightCycler Red 640 attached to 5' terminus; ph: 3' phosphate). The donor probe was complementary to the wild-type sequence. During melting curve analysis, a more stable duplex with the wild-type allele than with the mutant allele was formed, resulting in an allele-specific melting curve (62.5 °C vs. 56 °C).

**Melting curve analysis:**



### **DNA Sequencing:**

DNA sequencing was performed using Sanger's dideoxy DNA with fluorescently labelled dideoxy nucleotide triphosphates using the Big Dye Terminator cycle sequencing kit V 1.1 or V3.1 (Applied Biosystems, Foster City, CA) in an automatic sequencer ABI 310 (Applied Biosystems, Foster City, CA). The PCR products were purified using a PCR clean up system (Millipore, Bedford, MA) and cycle sequencing reaction was set up using the purified product. After the cycle sequencing reaction, the product was again purified using post PCR clean up kit (Millipore, Bedford, MA) and loaded in the genetic analyzer. All the nucleotide changes identified were confirmed by repeating the PCR, CSGE and sequencing reactions.

#### **( i ) SPINK 1 mutations (N34S, P55S):**

PCR was performed on Exon3 of SPINK 1 gene. N34S and P55S mutations were identified after sequencing the PCR product in ABI310 genetic sequencer.

#### **( ii ) PRSS1 MUTATION (N29I & R122H) :**

Cationic trypsinogen gene (PRSS1) mutations like R122H and N29I were analysed using PCR and direct DNA sequencing.

#### **( iii ) PRSS2 mutation (G191R)- Real time detection in Cohort-I:**

A dual-colour allele-specific discrimination assay for genotyping the exon 4 mutation at codon 191 of the *PRSS2* gene using the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories) with molecular beacons (figure III).

( iv) PRSS2 mutation(G191R)- Melting curve analysis for Cohort II: We performed melting curve analysis for the G191R alteration in cohort II using a pair of fluorescence resonance energy transfer (FRET) probes and the LightCycler (Roche Diagnostics).



**Cathepsin B gene:**

The exon 3 and Exon 4 and the exon intron boundary of cathepsin B was analysed using the oligonucleotides as shown in Table 9.1. Amplified PCR products were subjected to direct DNA sequencing using ABI310 genetic analyzer

**CONSENT :**

Informed written consent (Annexure1) was taken for blood collection and DNA analysis prior to the study. Peripheral blood sample (10 ml) was collected from the antecubital vein using K3EDTA coated vacutainer tubes (VACUTTE). This blood was stored at -20 degree celsius . Genetic studies were performed at Wellcome Research Laboratory, CMC, Vellore.

**STATISTICAL ANALYSIS:**

Data was analysed by statistical software SPSS (Statistical Package for Social Sciences, release 11.0, standard version; SPSS Inc).

This was a retrospective descriptive study and data was reported as means with standard deviation or median with ranges for continuous variables and as frequencies and percentages for categorical variables.

For continuous variables , t test was used for normally distributed variables and Mann Whittney test for non normally distributed variables. The results between groups were subjected to Chi-Square tests to assess for significance of genetic mutations and treatment modalities. A p value of less than or equal to 0.05 was considered statistically significant.

## RESULTS

### Age Distribution:

The mean age of patients was 32.6 years (13.9SD), the youngest being 6 years and the eldest 76 years .

There were 84 (44.6%) patients below 30 years and 104 (55.3%) above 30 years. Mean age of males was 34.0 years and females 29.3 years

### Sex Distribution:

132 (70.2%) patients were males and the rest 56(29.8) females , the male: female ratio being 2.3 : 1 .

32 females were < 30 years and 24 were > 30 years of age.

52 males were < 30 years while 80 were > 30 years of age.

### Geographic Distribution:

126 ( 67.02% ) were from the East of India ; 44 patients ( 23.40%) from South and 18 ( 9.57% ) from North of India, reflecting patient profile visiting CMC.

### Number of episodes of pancreatitis:

The number of episodes of recurrent acute pancreatitis in patients is shown in the Table : I .Majority of the patients (38.3%) had 2 episodes while 4 had 10 episodes of pancreatitis. The mean number of attacks were 3.53.

Table I. Episodes of pancreatitis

Episodes of pancreatitis	No: of patients(%)
2	72(38.3%)
3---4	58(30.9%)
5—6	49(26.1%)
8—10	9(4.7%)

### Symptoms:

All patients presented with abdominal pain ;159 patients (84.6%) had vomiting  
72 patients (38.3 % ) had constipation and jaundice was seen in 28(14.9%) patients.

### Laboratory Tests:

Serum calcium was estimated in 185 patients. 5 patients ( 2.7 %) had calcium levels more than 10.5 mg /dL. Serum cholesterol and triglycerides were done in 175 patients . 6 (3.2%) patients had triglyceride levels higher than 500 mg/dl.

### Biliary Microliths :

60 (31.9%) patients had sludge in gall bladder or microlithiasis in the bile aspirated either from bile duct or from the duodenum adjacent to the ampulla.

### **Etiology:**

Etiology of RAP is shown in Table II.

Table II. Etiology

Etiology	Number	%
Biliary	90	47.9
----Gall stone/CBD stone	38	20.2
----Sludge/microlithiasis	52	27.7
Structural	29	15.4
----Pancreas divisum	17	9.1
----Papillary stenosis	6	3.1
----Anomalous pancreato biliary	3	1.6
----Suprapapillary duodenal diverticulum	3	1.6
Alcohol	20	10.6

Metabolic	11	5.9
----Hypercalcemia	5	2.7
----Hypertriglyceredemia	6	3.2
Trauma	3	1.6
Idiopathic	34	18.1
Ascariasis	1	0.5
Total	188	100

Region wise distribution of etiological factors in RAP are shown in Table III.

Etiological profile were grossly similar in patients from East,South and North of India.

Table III. Etiology (Region wise)

Etiology	East (n-126)	South (n-44)	North (n-18)
Biliary	62 (49.2 %)	21( 47.65 %)	7 (38.89 %)
Alcohol	10 (7.93 %)	8 (18.18 %)	2 (11.11 %)
Idiopathic	24 (19.4 %)	5 (11.36 %)	5 (27.78 %)
Others	30 (23.8 %)	10 (22.73 %)	4 (22.22 %)

The most common etiology was biliary in 90 patients(47.9%) which included gall stones,CBD stones gall bladder sludge and microlithiasis.Structural causes like pancreas divisum, papillary stenosis, anomalous pancreato-biliary union and suprapapillary duodenal diverticulum were seen in 29 patients (15.4%).Metabolic causes like hypercalcemia and hypertriglyceredemia were seen in 11 patients(5.9%). 34 patients (18.1%) in whom no etiological factor was identified were labelled Idiopathic. Multiple etiological factors were detected in 24(12.8%) of patients.

Details are shown in Table 1V.

Table IV: Multiple etiologies.

Etiologies	No.of patients	Therapy	Response / No response
Microliths + PD	1	Sphincterotomy.	Response
Microliths + PS	1	Sphincterotomy.	No response
Microliths + HTGL	1	Niacin	Developed CP
Microliths + Alcohol	5	Sphincterotomy(2) UDCA (2)           Lost to FU (1)	Response (1) Response (2)
Alcohol+HTGL	1	Lost to FU	-
Alcohol+gallstones	6	Sphincterotomy and stone extraction(4) Conservative (2)	Response (4) On FU
PD + gall stones	3	Bil.sphincterotomy(3)	Response (2) No response(1)
Hypercalcemia+PD	1	Antioxidants	On FU
Hypercalcemia + gall stones	1	Conservative	On FU
Hypercalcemia +PD+gall stones	1	Bil sphincterotomy	Response
APBU+PD	1	Bil sphincterotomy	Response
APBU+microliths	1	UDCA	Response
APBU+choledochal cyst	1	Surgery	Response.

(PD:pancreas divisum,PS:papillary stenosis,APBU:anomalous pancreatic biliary  
union,FU:follow up,HTGL:hypertriglyceridemia)

**Complications:**

Complications are shown in Table V.

Table V. Complications:

Complications	Number	%
Pseudocyst	14	7.4
Necrosis	3	1.6
Abscess	3	1.6
Pseudoaneurysm	1	0.5
Venous thrombosis		
Splenic Vein thrombosis	8	4.3
Superior Mesentric Vein thrombosis	3	1.6
Portal Vein thrombosis	1	0.5

Most common complication was pseudocyst seen in 14 patients (7.4%). Thrombosis of portal venous system was seen in 12 patients (6.4%)

**TREATMENT AND RESPONSE:****Drug Therapy:**

Patients detected to have microlithiasis were given a trial of ursodeoxycholic acid for 6 months. A subset of patients with idiopathic RAP were also given a trial of UDCA to assess response. Table V1 shows details of response to UDCA therapy. 76 % of patients with microlithiasis and 40 % of patients with idiopathic RAP responded to UDCA.

Thirteen patients diagnosed as idiopathic RAP were given an empiric trial of pancreatic enzyme therapy suspecting early chronic pancreatitis. Details of response are shown in the Table VI . 39% of patients with idiopathic recurrent

pancreatitis responded to pancreatic enzyme supplements. Duration of follow up ranged from 6 months to 4 years.

Table-V1. Medical therapy.

Drug	No.	Responded to therapy(%)	No response	Lost to follow up
Ursdeoxycholic acid				
Microlithiasis	41	31(75.6)	8	2
Idiopathic	10	4(40)	4	2
Pancreatic enzymes				
Idiopathic	11	5(38.5)	3	5

### **Endotherapy:**

Biliary sphincterotomy and stone extraction was performed for patients with choledocholithiasis. Biliary sphincterotomy was performed for patients with microlithiasis responding to a trial of UDCA, those with papillary stenosis and a subset of patients with idiopathic RAP. Accessory papillary sphincterotomy was performed for patients with pancreas divisum. Details of response to endotherapy are shown in the Table VII.

67% patients with microlithiasis and 50 % of idiopathic RAP responded to biliary sphincterotomy. Majority of patients with pancreas divisum (73%) responded to accessory papilla sphincterotomy.

Table VI1. Endotherapy.

Endotherapy	Number	Response(%)	No response	Lost to follow up
Biliary sphincterotomy				
Microlithiasis/sludge	36	24(66.7)	9	3
Papillary stenosis	2	2(100)	-	-
Idiopathic	12	6(50)	3	3
Accessory Papilla sphincterotomy				
Pancreas divisum	11	8(72.7)	3	-

### **Surgery:**

Cholecystectomy was performed in patients with gallbladder stones.

Necrosectomy was performed in 3 patients and pseudocyst drainage in 6 patients.

### **Follow Up:**

Mean follow up period was 3 years ; 56 patients (29.8%) were lost to follow up.

23 patients (12.2% ) developed chronic pancreatitis during follow up-

14 patients within 1 year of presentation ; 5 patients in 2 years ; 2 in 3 years

and one each in 4 and 5 years,. 9 (39.1%) of idiopathic pancreatitis, 5 (21.7%)

with pancreas divisum , 4 ( 17.3%) with biliary microliths , 2 (8.7%) with

hypercalcemia and 1 (4.34%) each with alcohol , papillary stenosis and

hypertriglyceridemia progressed to chronic pancreatitis.



**Mortality:**

27 (14.36% ) patients had severe pancreatitis .

The etiology among these patients were Idiopathic pancreatitis in 10 (37.04%) , biliary(7 gall stones and 3 microliths ) in 10 (37.04%) patients, alcohol , pancreas divisum in 3 (11.1%) each , and papillary stenosis in 1 (3.7%) patient .

4(15%)developed chronic pancreatitis (idiopathic :3 , pancreas divisum:1)

There was no mortality in the group of patients studied.

**GENETIC STUDIES:**

Genetic analysis done in a subset of 17 patients is shown in Table VII1.

Table VII1:

REGION	POLYMORPHISM	PATIENTS (n - 17)	CONTROLS ( n – 150)	SIGNIFICANCE
Exon 3	PRSSI (R122H)	0	0	-
Exon 2	PRSSI (N29I)	0	0	-
Exon 4	PRSS2 (G191R) (+/-)	0	3(2.2%)	
Exon 3	SPINK1((N34S) (+/-)	0	6(4.5%)	0. 62
Exon 3	SPINK1 (N34S) (+/+)	0	1(0.7%)	
Exon 3	SPINK1 (P55S) (+/-)	0	3(2.2%)	0.77
Exon 3	SPINK1 (P55S) (+/+)	0	1(0.7%)	

Cathepsin B genotypes:

Exon	POLYMORPHISM	PATIENTS	CONTROLS	SIGNIFICANCE
Exon 3	<u>C12T (+/-)</u>	0	28 (20.9%)	<u>0.04</u>
Exon 3	C76G (+/-)	8 (47.1%)	58 (43.3%)	0.76
Exon 3	C76G (+/+)	1 (5.9%)	16 (11.9%)	
Exon 3	T663C (+/-)	1(5.9%)	30 (22.4%)	0.13
Exon 3	T663C (+/+)	0	8 (6%)	
Exon 3	C173G (+/-)	0	1 (0.7%)	1.0
Exon 3	C182T (+/-)	0	1 (0.7%)	1.0
Exon 3	G205C (+/-)	0	0	
Exon 3	T232G (+/-)	0	0	
Exon 3	C261T (+/-)	0	2 (1.5%)	1.0
Exon 3	G265T (+/-)	0	3 (2.2%)	1.0
Exon 3	G274C (+/-)	0	3 (2.2%)	1.0
Exon 3	G394A (+/-)	0	6 (4.5%)	1.0
Exon 3	A335T (+/-)	0	33 (24.6%)	<u>0.05</u>
Exon 3	A335T (+/+)	0	4 (3%)	
Exon 3	C412T (+/-)	0	1 (0.7%)	1.0
Exon 3	G426C (+/-)	0	0	
Exon 3	G460C (+/-)	0	1 (0.7%)	1.0
Exon 3	A505T (+/-)	0	1 (0.7%)	1.0
Exon 3	G513A (+/-)	0	2 (1.5%)	1.0
Exon 3	G575T (+/-)	0	2 (1.5%)	1.0
Exon 3	C595T (+/-)	0	1 (0.7%)	1.0
Exon 3	A608C (+/-)	0	1 (0.7%)	1.0
Exon 3	G642C (+/-)	0	1 (0.7%)	1.0
Exon 4	A790G (+/-)	3 (17.6%)	13 (9.7%)	0.57
Exon 4	A790G (+/+)	0	1 (0.7%)	-
Exon 4	G834C (+/-)	1 (5.9%)	0	-

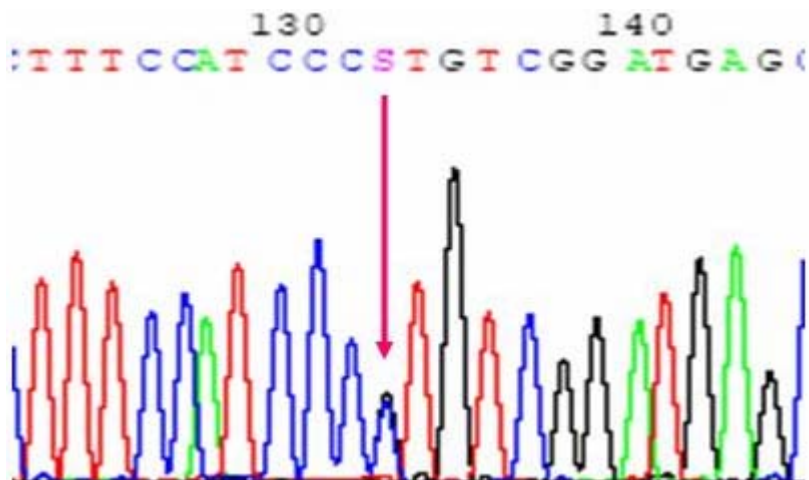
None of the patients showed mutation in PRSS1 (R122H ; N29I),PRSS2 (G191R) and SPINK1 (N34S ; P55S) genes.

Few control subjects showed heterozygous and homozygous mutations in SPINK1 (N34S and P55S ) and PRSS2 (G191R) genes.

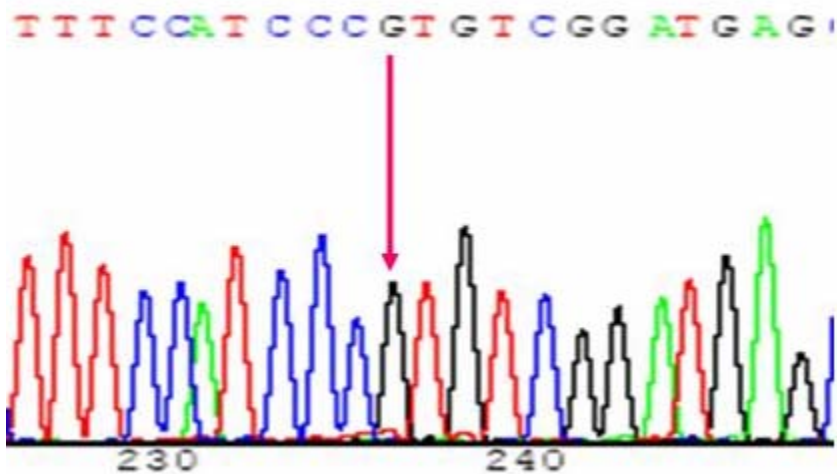
Analysis of cathepsin B gene showed 4 polymorphisms in patients with RAP. (Exon 3:C76G, T663C; Exon 4 : A790G, G834C ) and 18 polymorphisms in the controls.(Table VII). C12T and A335T polymorphisms on Exon 3 were significantly more common in controls as compared to patients.

## CATHEPSIN B GENE ELECTROPHEROGRAM:

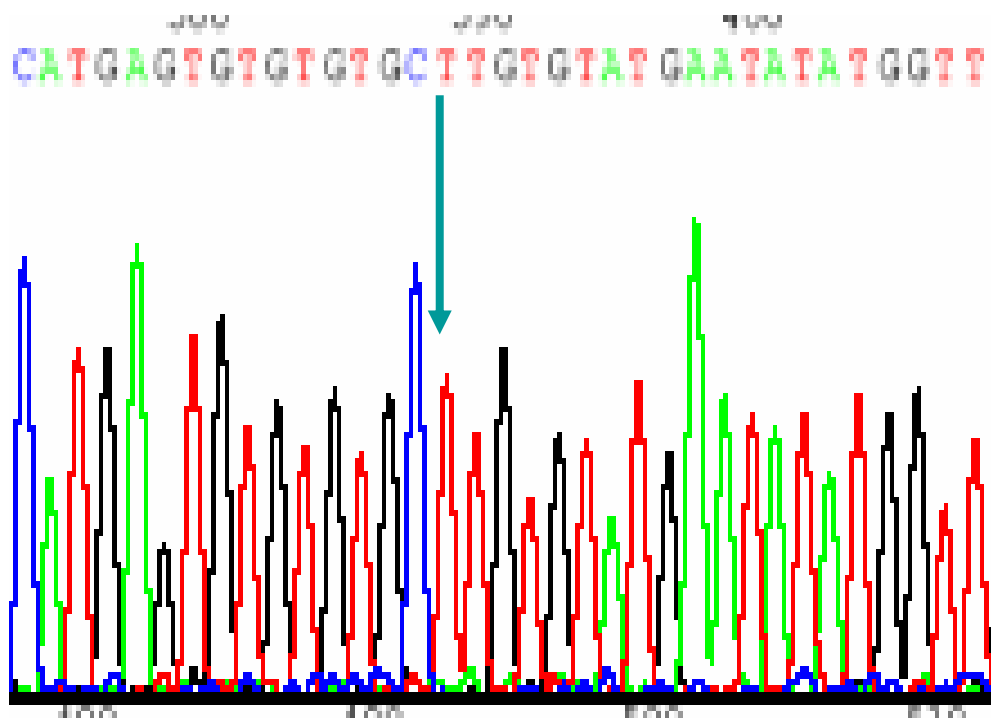
1. C76G (+/-)



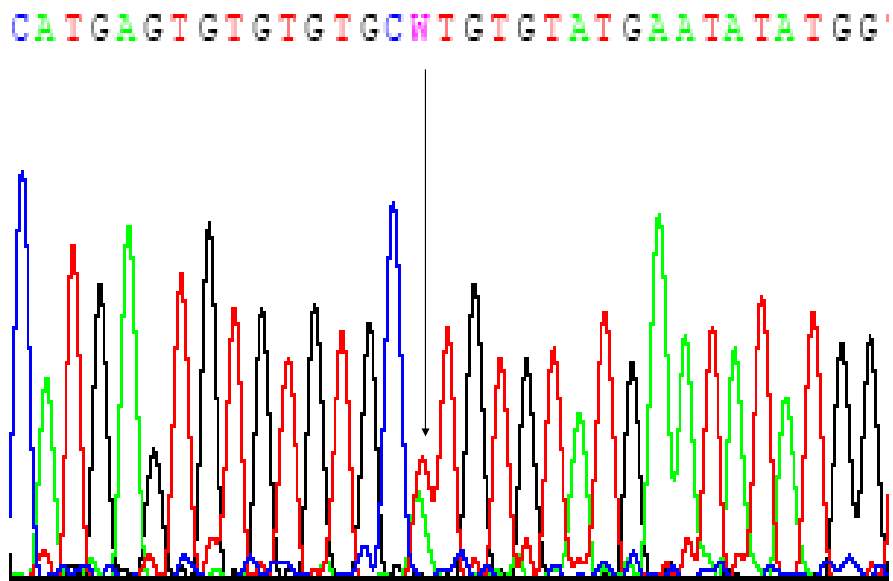
2. C76G (+/+)



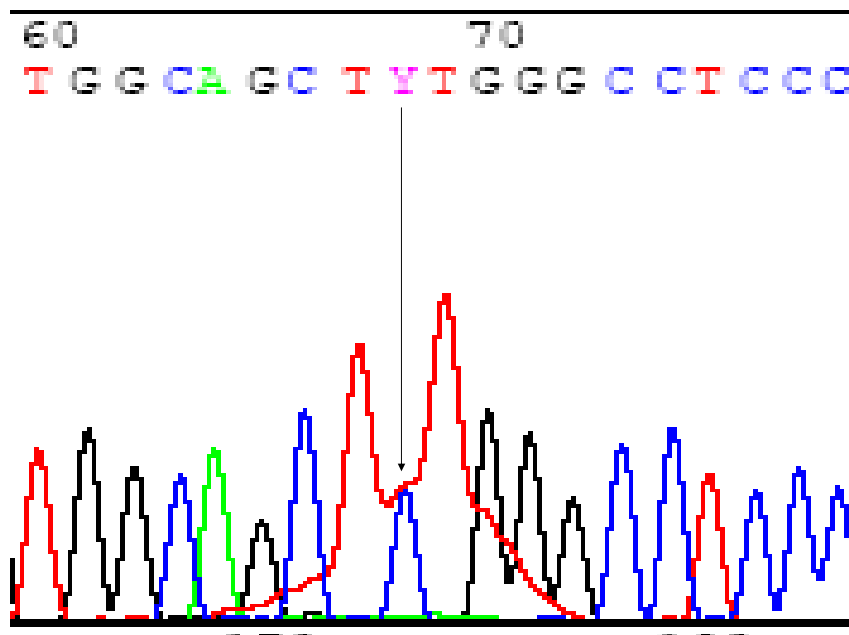
3. A335T(+/+)



4. A335T(+/-)



5. C12T(+/-)



## DISCUSSION

The current study is a retrospective analysis of 188 patients diagnosed to have RAP over a 5 year period. Numerous studies have been published on acute and chronic pancreatitis , but few have focused on recurrent acute pancreatitis. RAP therefore still remains a clinical and diagnostic problem.

### Demography:

The mean age of patients was 32.6 years (13.9SD), the youngest being 6 years and the eldest 76 years . Mean age of patients in the studies done by Wang et al <sup>(9)</sup> and Gullo et al <sup>(11)</sup>, were 41 years and 43 years respectively. The slightly older age of presentation in the above studies may be due to alcohol etiology in majority of patients. Majority of the patients( 70.2%) were males which was similar to the studies by Wang (63%) and Gullo (78.8%). 57 % of females were less than 30 years of age while 60.6 % of males were more than 30 years of age.

Majority of the patients ( 67%) were from Eastern parts of India ; 23.4% from South and 9.6 % from North India. This reflects the profile of patients visiting CMC for treatment and suggests that RAP is seen all over the country.

72 patients (38.3%) had relapsed twice, while rest of them had more than 2 episodes. This was similar to the study of Garg <sup>(117-119)</sup> et al but differed from the study by Gullo et al who showed that 70.7% of patients with alcoholic RAP and 80.6% with gall stones had 2 attacks.

The most frequent symptom was abdominal pain (100%) followed by vomiting (84.6 % ) and ileus (38.3 % ) .which was identical to other studies .



### Aetiology:

The most common aetiology was biliary seen in 90 patients ( 47.9% ) which included gall stones , CBD stones , gall bladder sludge and microlithiasis . 52 (27.7%) had biliary microliths / sludge and 38 (20%) biliary stones. Region wise analysis showed similar frequency of biliary pancreatitis in East , South and North India.(East : 49.2%; South : 47.5%; North : 38.9%). This is surprising because Tandon <sup>(118,119)</sup> found that North Indians and Bengalis have 2-4 fold higher prevalence of gall stones compared to South Indians. Our data differs from Western data where alcohol was the most frequent cause of RAP seen in 57 % of patients . 20 (10.6%) patients in the current study had alcohol related RAP. Biliary disease and alcohol had a combined frequency of 58.5 % which was lower than Western data where the combined frequency was about 70%. <sup>(11)</sup>

Structural abnormalities of the pancreas were seen in 29 patients (15.4%). Pancreas divisum was the most common structural abnormality seen in 9%. This is lower than the Western data where pancreas divisum is implicated as a cause of pancreatitis in 20% of the patients <sup>(125)</sup>. Metabolic etiology was seen in 11 patients 5.9%); 5 patients had hypercalcemia and 6 hyperlipidemia. No etiology was detected in 34 patients (18.1%) and they were labelled as idiopathic RAP . This is similar to the published data where 10-25 % of patients with RAP have no identifiable cause and are termed idiopathic. Zhang et al <sup>(10)</sup> studied 77 RAP patients and found 48 (62.33%) had biliary pancreatitis,

3 (3.89%) had alcohol-induced pancreatitis, 3 (3.8%) had hyperlipidemic pancreatitis, and 21 (27.27%) had idiopathic pancreatitis.

Region wise distribution of etiological factors were grossly similar in patients from East, South and North of India.

Multiple etiological factors were detected in 24(12.8%) of patients. Deciding which factor is responsible for RAP may sometimes be difficult. Response of the patient to empirical therapy directed towards the probable cause confirms the cause of pancreatitis.

#### Morbidity and Mortality:

14 (7.4%) patients had pseudocysts, 12 (6.4%) had venous thrombosis, 3 (1.6%) had pancreatic necrosis and pancreatic abscess. One patient had hemorrhage into the pseudocyst confirmed and treated by angiography.

Wang <sup>(9)</sup> noted several complications which in descending order of frequency, were pancreatic pseudocyst, pancreatic ascites and bacterial peritonitis, pulmonary infections, multiple organ failure and shock.

There was no mortality in the patients studied. In the study by Gullo et al <sup>(11)</sup> 5.9% died, all of whom had necrotizing pancreatitis. Most of the deaths occurred during the second attack of pancreatitis. They also found that the morbidity in necrotizing recurrent alcoholic pancreatitis was significantly lower than in patients with necrotic biliary (30%;  $p < 0.0002$ ) and necrotic idiopathic pancreatitis (25%;  $p < 0.04$ ). In the present study severe acute pancreatitis was detected more often in biliary and idiopathic pancreatitis (37%) as compared to alcoholic pancreatitis (11%).

Absence of mortality in our study could be because most of our patients (85.6%) had mild pancreatitis and only 14.4% had severe pancreatitis requiring

prolonged hospital stay and surgical treatment.

#### Medical Therapy:

Therapy directed at specific underlying cause will reduce recurrence rate of pancreatitis. Ursodeoxycholic acid (UDCA) may help to dissolve the cholesterol crystals (microliths ) and prevent recurrent attacks of pancreatitis. Three therapies have been used in relapsing acute and chronic idiopathic pancreatitis – antioxidants, pancreatic enzymes and octreotide<sup>(120)</sup>

In a small study of pancreatic enzyme therapy, 12 patients with RAP had response rate (>50% reduction in frequency of attacks) of 75% . There is only a single trial using antioxidants in 5 patients with RAP. No conclusions can be drawn from this study because of the small number of patients. There is no data using octreotide in RAP. Conclusions on the efficacy of above therapy are difficult because of lack of large, placebo controlled trials with long term follow up.<sup>(120)</sup>

Details of response to therapy to UDCA in patients with microlithiasis and response to pancreatic enzyme therapy in idiopathic RAP are shown in Table V. 41 patients detected to have microlithiasis were given a trial of UDCA for 6 months. 31 (76%) had relief of symptoms , 8 had no response and 2 were lost to follow up . These data confirm the results of other studies that microlithiasis is a frequent cause of recurrent acute pancreatitis. <sup>(21,118)</sup>. The reason for recurrent pancreatitis in those on UDCA may be due to calcium bilirubinate crystals in bile, inadequate dosage of UDCA or irregular therapy.

All the 31 patients who responded to UDCA underwent biliary sphincterotomy as maintenance therapy is required to prevent recurrent stone formation.

13 patients diagnosed as idiopathic RAP were treated with pancreatic enzyme therapy and followed up for 6 months to 4 years. 5 (38.5%) responded, 3 had no response and 5 patients were lost to follow up. 10 patients with idiopathic RAP were treated with UDCA in the hope that undiagnosed microliths may be responsible for RAP in some of these patients. 4 (40%) responded, 4 had no response and 2 were lost to follow up. The numbers are small and no conclusions can be reached.

#### Endotherapy:

There is evidence to suggest that pancreatic duct obstruction can lead to acute pancreatitis. Rationale of endoscopic therapy is to relieve obstruction and prevent further episodes of pancreatitis. Endoscopic therapy has been shown to be beneficial in patients with RAP due to choledocholithiasis, biliary microlithiasis, sphincter of Oddi dysfunction, pancreas divisum, choledochocoele and ampullary tumors. There is however a paucity of controlled data regarding outcomes in patients undergoing endoscopic therapy in RAP.

Biliary sphincterotomy and stone extraction was performed for all patients with choledocholithiasis. Biliary sphincterotomy was performed on 36 patients with biliary sludge/microliths including 31 patients who responded to UDCA. 24 (66.7%) responded, 9 had no response and 3 were lost to follow up. This is similar to data from other studies with a response rate of 67 to 100 percent. <sup>(108,121)</sup>

Biliary sphincterotomy was performed in 12 patients with idiopathic RAP. 6 (50%) responded, 3 had no response and 3 were lost to follow up. These data suggest that patients who had response to biliary sphincterotomy may have occult microlithiasis

or sphincter of Oddi dysfunction. Sphincter of Oddi manometry was not performed in this study . 2 patients with papillary stenosis had good response to biliary sphincterotomy.

Accessory papilla sphincterotomy was performed in 11 patients . 8 (73%) responded while 3 continued to have recurrent attacks of pancreatitis .These results are comparable to data from other studies which show that endoscopic therapy of pancreas divisum decreases the rate of recurrence of pancreatitis in 70-90 % of patients when followed up for 5 years <sup>(122-124)</sup>

#### Surgery:

14 patients with gall stones had cholecystectomy, 3 patients had cystogastrostomy and 3 necrosectomy and pseudocyst drainage.

#### Chronic Pancreatitis on Follow Up:

Patients initially diagnosed to have RAP ,may develop chronic pancreatitis. One reason could be the low sensitivity of ERCP to diagnose early chronic pancreatitis. Pancreatic function test (secretin stimulation) and EUS may be more sensitive to detect early chronic pancreatitis. Another possibility could be development of chronic pancreatitis following recurrent attacks of acute pancreatitis.

23 (12.2%) patients developed chronic pancreatitis in 1 - 5 years during follow up. Initial diagnosis of these patients were as follows: Idiopathic pancreatitis: 9 (39%) , pancreas divisum : 5 (22%) , biliary microliths : 4 (17%) , hypercalcemia : 2 (9%) , alcohol, papillary stenosis , hypertriglyceridemia : 1 (4%) each.

### Does Idiopathic RAP really exist?

After extensive evaluation including history , laboratory tests , imaging ( ultrasound / CT scan) , ERCP and bile for microlithiasis , no etiology could be detected in 34 (18 %) patients. and they were labeled as Idiopathic RAP. 11 (32%) responded to empirical trial of UDCA or biliary sphincterotomy and had no relapses for at least one year suggesting that these patients may have occult microlithiasis or SOD. 9 (26%) developed chronic pancreatitis on follow up of 1-5 years . 11 (32 %) were lost to follow up . The above data suggests that extensive evaluation including bile microlith analysis , ERCP , SOM , EUS , empirical therapy for occult microlithiasis and follow up ( diagnosed chronic pancreatitis ) will detect a cause for RAP in almost all patients(93%).

### Genetic Studies :

Genes that are implicated in pancreatitis are PRSS1, PRSS2 , SPINK1 , CFTR and Cathepsin B. Discovery of these genetic mutations have not only provided insight into the molecular mechanisms of pancreatitis but also presents the possibility of a powerful diagnostic tool.

Several cases have been reported where patients with CFTR gene mutation without cystic fibrosis present with acute recurrent pancreatitis . These patients have compound heterozygosity in CFTR gene involving CFTR dele 2,3,(21 kb). These patients have congenital bilateral absence of vas deferens , normal sweat chloride concentration and no nasal polyps.<sup>(126)</sup> Though most studies on cationic trypsinogen and SPINK1 mutations have been in chronic pancreatitis , few studies have detected these mutations in acute pancreatitis .<sup>(3,6)</sup>

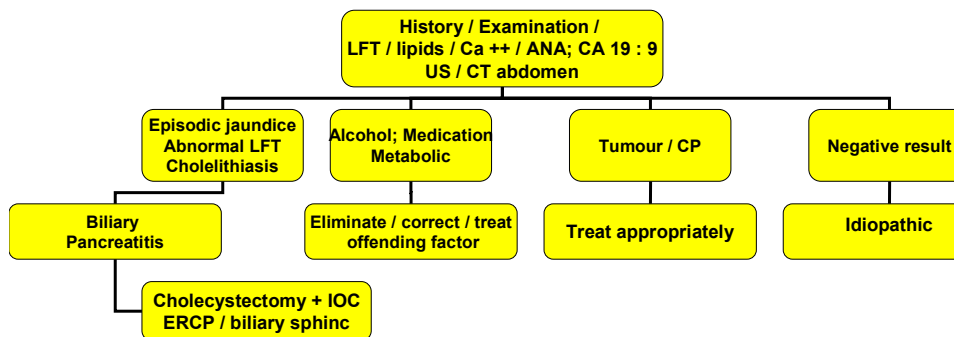
We analysed known mutations in PRSS1 gene (R122H,N29I) , PRSS2 gene (G191R),SPINK1 gene (N34S,P55S ) and polymorphisms in Exon 3 and 4 of Cathepsin B gene in a subset of 17 patients with RAP (Idiopathic :7 ; biliary :7 ; alcohol :2 ; papillary stenosis : 1 ) and 150 controls. None of the patients showed mutations in PRSS1 ,PRSS2 ,and SPINK1 genes. Analysis of Cathepsin B gene showed two polymorphisms in Exon 3 (C76G , T663C) and two in Exon 4 ( A790G, G834C) in patients with RAP and 18 polymorphisms in controls. C12T and A335T polymorphisms in Exon 3 were significantly more common in controls as compared to patients . C76 G heterozygosity was seen in 5/7 patients with Idiopathic RAP and 3/7 patients with biliary RAP.

Our study suggests that gene mutations do not play a role in aetiopathogenesis of RAP. Definite conclusions are not possible due to small sample size studied .Large sample size and long term follow up are required to address the role of genetics in RAP and genotype phenotype correlations .

A suggested algorithm for the evaluation and management of RAP .

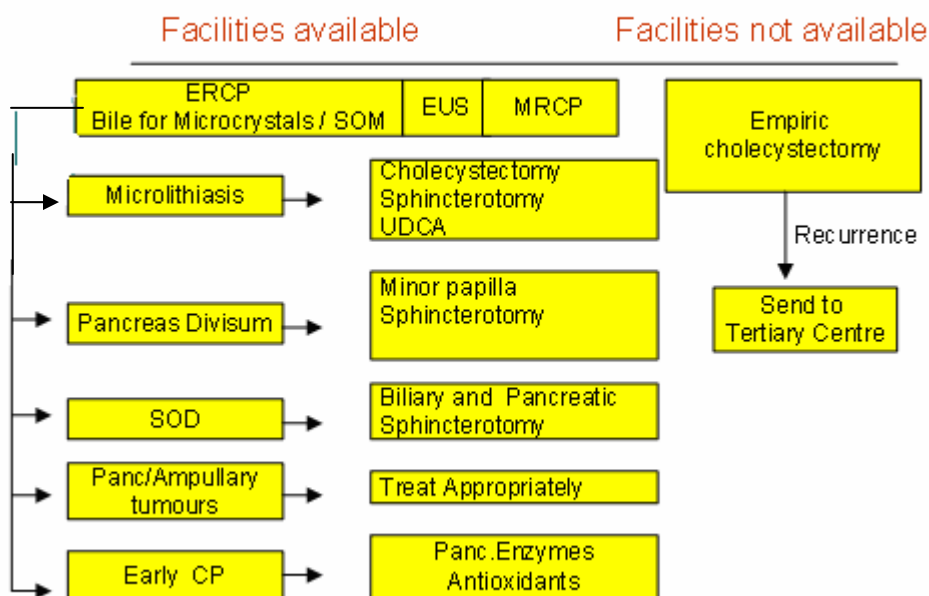
Step I:

## Initial evaluation & management of Recurrent Acute Pancreatitis



Step II.

## Specialised evaluation & management of IRAP





## CONCLUSIONS:

1. Biliary disease (stone (20%); sludge / microlithiasis (28%) is the most common cause of recurrent acute pancreatitis (RAP).
2. Determining etiology is important to assess the progressive nature of the disease .  
The extent of evaluation impacts the frequency with which an etiology can be found.  
Bile for microliths and ERCP should therefore be part of evaluation of RAP as these detect the etiology in 1/3<sup>rd</sup> of patients.
3. Distribution of etiological factors was similar in East , South and North India.
4. After extensive evaluation including ERCP and testing bile for microliths , a cause for RAP could not be detected in 18% of patients.
5. Genetic mutations do not play a major role in the etiopathogenesis of RAP.  
Further studies with larger sample size needs to be done.
6. Severe pancreatitis (14%) was predominantly seen in biliary and Idiopathic RAP.
7. Patients with biliary microlithiasis responded well to treatment with UDCA and biliary sphincterotomy.
8. Patients with pancreas divisum responded well to accessory papilla sphincterotomy.
9. Some patients labeled as Idiopathic RAP may respond to empiric biliary sphincterotomy as they may have occult biliary microlithiasis or sphincter of Oddi dysfunction.
10. Patients (38%) labeled as Idiopathic RAP responded to empiric trial of pancreatic enzymes. Large controlled trials are required to determine the role of

pancreatic enzymes , antioxidants or octreotide in therapy of Idiopathic RAP.

11. Does Idiopathic RAP really exist ?

An etiology was obtained in 93% of patients after extensive evaluation , empiric biliary sphincterotomy in Idiopathic RAP and development of chronic pancreatitis on follow up. It is possible that on further follow up , the 7 % of patients may progress to chronic pancreatitis.

12. Follow up of patients with idiopathic RAP is necessary as some may progress to chronic pancreatitis.

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### **Consent Form**

It has been explained clearly to me in a language that I understand:

- i) that a research study on genetic mutations in recurrent acute pancreatitis is being conducted in the Department of G.I. Sciences, C.M.C.H., Vellore.
- ii) Information obtained from the study will be kept confidential.
- iii) A sample of 10ml of blood will be collected for this purpose.

I hereby give my full consent to give my blood sample for the above mentioned study.

Dr. Sajith K.G

Patient's name:

Registrar,

Hospital number:

Dept. of GI Sciences,

C.M.C.H., Vellore.

Signature:

## P R O F O R M A

Serial Number

Name:

Age

Sex

Hosp.No.

Address

Date of Diagnosis

Final Diagnosis

Number of episodes:

Number of Hospitalisations:

Scoring system for severity – Ranson’s criteria

Mild / Severe

Clinical Data

Complaints

Abdominal Pain

Duration

Location

Intensity

Character

Nausea

Vomiting

Constipation/Obstipation

Constitutional symptoms:

Fever

Anorexia

Weight loss

Others

Complications

Local - Necrosis

Abscess

Pseudocyst

Ascites

Pleural effusion

Systemic -     Hypotension  
  
                    Respiratory failure  
  
                    Renal Failure  
  
                    GI bleed

Physical examination

Weight	Height	BMI
Pulse	BP	
Pallor	Icterus	Cyanosis
Clubbing	Lymphadenopathy	Edema
Echymosis in flanks or periumbilical region	Thrombophlebitis	
Polyarthrititis	Subcutaneous nodular fat necrosis	

Systemic examination

Abdomen	Abdominal distension
	Organomegaly
	Tenderness
	Bowel sounds
	Free fluid

RS:	CVS:	CNS:
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Past History

Personal History	Alcohol (Duration / Type - Brand / Intake per day)
	Smoking (Duration/ Type – Cigarette/ Beedi / No. per day)

Treatment History

Drug History

Trauma

Family History

Investigations

Complete Blood Counts:

Biochemistry – LFT

RFT

Amylase/ Lipase

Calcium

Lipid Profile

RBS

LDH

CA 19-9

VIROLOGY -- HBsAg / HCV/ HIV

ANA

ESR

RADIOLOGY

Chest x- ray

Abdominal x-ray

Ultrasound

CT Scan

ENDOSCOPY

ERCP

GENETIC ANALYSIS :

PRSS 1

R122H

N291

PRSS 2

SPINK 1

N34S

P55S

Cathepsin B